Responses of Fungal Hyphae to Soil Fungistasis

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ABSTRACT

Hyphae of 20 different fungi were incubated for 4 hr on cellophane placed on natural soil amended with different amounts of alfalfa extract. Linear growth, expressed as per cent of growth occurring on alfalfa extract agar, was plotted against log concentration of alfalfa extract. The area under the curve, taken as per cent of the total area, was termed the "relative growth index". Relative growth indices varied from 20.3 to 80.2. A high relative growth index indicated low sensitivity to soil fungistasis. Relative growth indices were directly correlated with hyphal growth rate on alfalfa extract agar ($r = 0.91$), and with hyphal diameter ($r = 0.82$). Hyphal diameter was also directly correlated with growth rate ($r = 0.88$) and spore volume ($r = 0.84$). Fungistasis sensitivity indices of spores and relative growth indices of hyphae were inversely correlated ($r = 0.74$). Lysis, as determined by loss of radioactivity from ^{14}C-labeled hyphae on soil, was delayed when alfalfa extract was added to soil. Fewer than 10% of the hyphae of any of 10 fungi incubated on membrane filters on natural soil remained viable after 8 days. The conidial portion of germinated spores of five species with multicellular spores survived longer than the hyphae themselves, whereas conidia of five species with single-celled spores survived no longer than their hyphae. Some of the original, germinated conidia of *Helminthosporium sativum* and *H. victoriae* were able to regerminate at least 5 successive times on an acidified agar medium following complete lysis of germ tubes on soil. Phytopathology 61: 1355-1362.

Additional key words: lysis of fungal hyphae in soil, spore germination in soil, *Glomerella cingulata*.

Numerous papers have dealt with the restriction of germination of fungal spores in soil, but less attention has been given the inhibition of hyphal growth in soil. Mycelia of *Helminthosporium sativum* (7) and *Trichothecium roseum* (2) were less inhibited than spores when placed directly on soil. However, growth of hyphae of *Fusarium oxysporum* f. sp. *cubense* was more strongly inhibited than was spore germination in nonsterile soil extracts (9). Steiner & Lockwood (8) compared the fungistatic effect of soil on fungal mycelia and on spores, using mixtures of sterilized and natural soil. The ratios of sterile:natural soil for 50% germination of spores and for 30 μ new growth of mycelia were determined for eight fungi. They found a direct correlation between the response of spores and mycelia. However, mycelia were less sensitive to soil fungistasis than were corresponding conidia, and the range of sensitivity indices for mycelia (0.2-2.6) was much narrower than that for the corresponding conidia (0.2-23).

The purpose of the present research was to measure the sensitivity of hyphae of different fungi to fungistasis, and to identify factors related to differences in response.

MATERIALS AND METHODS.—*Maintenance of fungi.*—The following fungi were maintained on potato-dextrose agar (PDA): *Aspergillus fumigatus* Fresenius, *A. ustus* (Bainer) Thom & Church; *A. terreus* Thom; *Fusarium roseum* (Lk. ex Fr.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum'; *F. solani* f. sp. *pisi* (F. R. Jones) Snyd. & Hans.; *Glomerella cingulata* (Ston.) Spauld. & Schrenk; *Mucor ramannianus* Moeller; *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr.; *Neurospora tetrasperma* Shear & Dodge; *Penicillium frequentans* Westling; *P. variabile* Sopp; *Thielaviopsis basicola* (Berk. & Br.) Ferr.; *Trichoderma viride* Pers. ex Fr.; and *Verticillium albo-atrum* Reinke & Berth. *Alternaria tenuis* Nees ex Corda, *Botrytis cinerea* Pers. ex Fr., *Curvularia lunata* (Wakker) Boedijn, and *Stemphylium sarcinaeforme* (Cav.) Wiltshire were maintained on V-8 juice agar (per liter: 200 ml V-8 juice (Campbell Soup Co.), 20 g agar, 2 g $CaCO_3$). *Helminthosporium sativum* Pam., King & Bakke, and *H. victoriae* Meehan & Murphy were maintained on moist, sterilized wheat straw.

Preparation of alfalfa extract and soil plates.—Conover loam soil was used in all experiments, and possessed the following characteristics: pH, 6.7-7.1; organic matter, 3.8%; water-holding capacity, 42.7%; clay, 7.5%; silt, 42.8%; and sand, 49.7%. This soil, collected from a Michigan State University farm, was sieved and air-dried to 8-10% moisture content. Samples of 25 g dry soil were amended with 0.05, 0.1, 0.2, 0.5, 1, and 2 ml of alfalfa extract, and mixed thoroughly before adding water to bring the moisture content to 25%. The amended soil was placed in a plastic petri dish (45 x 10 mm), and a smooth surface was made.

Alfalfa extract was prepared by steaming 20 g dried alfalfa hay with 120 ml distilled water for 30 min. The extract was passed through Whatman No. 1 filter paper, then sterilized by autoclaving. In some experiments, soil was amended with the following concentration of a mixture of glucose and peptone, in μg/g soil: 200:100, 400:200, 2,000:1,000, 4,000:2,000, 8,000:4,000.

Assay of hyphal growth on soil.— Conidia collected from the cultures were washed in sterile distilled water 3 times by centrifugation. Washed spores were placed on noncoated, boiled cellophane discs 7 mm in diam on PDA. The cellophane discs were notched on one side to facilitate orientation.

For assaying hyphal growth, cellophane discs with

germinated spores were transferred to water agar when germ tubes were 2-3 times the length of the larger spores and 3-4 times the length of the smaller spores; 10-20 hyphae of approximately the same length were measured in each of at least two fields on each of duplicate plates for each fungus. A diagram showing the position and the length of each hypha was drawn. Then the cellophane discs were transferred to soil plates amended with alfalfa extract. After 4-hr incubation at 24 C, the hyphae were stained and killed by placing the discs on sand saturated with rose bengal solution (10). The original hyphae were identified with the aid of the diagram, and net growth was determined.

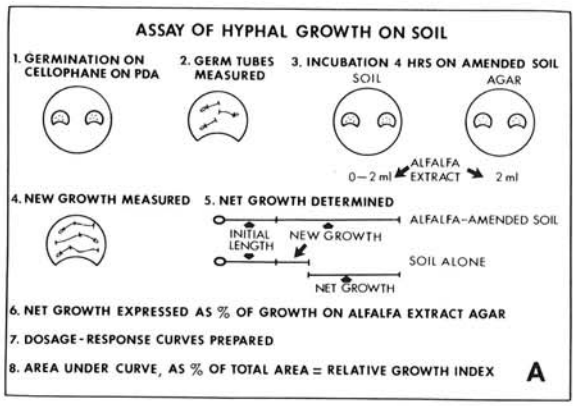
Net growth was determined by subtracting the initial length of the hypha and the growth on nonamended soil from the final length. To eliminate inherent differences in growth rates among the fungi, net growth of each fungus was expressed as per cent of growth of that fungus occurring on alfalfa extract agar (8 ml alfalfa extract in 0.1 *M* phosphate buffer in 100 ml water agar, pH 6.8). Growth on this medium was assumed to represent maximum growth for the fungus under noncompetitive conditions. These values were plotted against the log concentration of alfalfa extract in soil. The proportion of the area under each curve to that of the whole rectangle, expressed in per cent, was termed the "relative growth index". The assay procedure is outlined in Fig. 1-A.

Determination of hyphal diameter and growth rate.—Hyphal diameter was determined when hyphae were 2-8 times the length of the spores, by taking the mean of the tip and basal portions of the hyphae, measured microscopically (x970). Growth rate for the 2nd to 4th hr after germination was determined by the formula $\frac{\log_e L/l}{t}$, where L = final length of hypha, l = initial length, e = natural logarithm, and t = the time interval between initial and final length, i.e., 2 hr (4).

Nutrient analysis of amended soil.—Total soluble carbohydrates and glucose were extracted from nonamended soil and from alfalfa extract-amended soil at intervals after amendment. Twenty-five ml distilled water was added to each soil sample and shaken 5 min. The soil suspension was centrifuged at ca. 6,000 *g* for 10 min in a refrigerated centrifuge. The supernatant solution was sterilized by passage through a sterile Millipore filter (pore size 0.22 μ). Soluble carbohydrates were determined by the anthrone method (6). More than 90% of total soluble carbohydrates were recovered from amended soil in three water extracts. About 72% was recovered in a single extract, which was done routinely. Glucose was determined colorimetrically at 400 mμ with the Glucostat reagent (Worthington Biochemical Corporation), used according to the manufacturer's directions. Glucose at concentrations of 20, 40, and 80 μg/g was used as a standard.

Assay of hyphal lysis using ^{14}C-labeled hyphae.—Seventy-five μliters glucose ^{14}C (specific activity 200 mc/nM) was added to 1 liter of 0.5% unlabeled glucose and 0.2% peptone. Two ml of this liquid medium were inoculated with ca. 4,000 spores of each fungus. After incubation at 24 C for 12 hr, the labeled hyphae were washed thoroughly and placed on Nuclepore membranes (General Electric Company; pore size 0.5 μ), and incubated on nonamended natural soil or on soil amended with alfalfa extract. After incubation, the mycelia were dried in planchets. Radioactivity was determined in a gas-flow counter with Micromil window. At 0 time, radioactivity in the hyphae ranged from 200 to 500 counts/min. Radioactivity remaining in the lysed hyphae was expressed as per cent of that in control hyphae measured at 0 time. Lysis of hyphae from the same samples was estimated microscopically using a 0-6 scale (3).

Assay of hyphae survival.—Washed spores placed on Nuclepore membranes were incubated on PDA until germ tubes were 5-10 times the length of the spores. The positions of selected hyphae were diagrammed. Three replicates of 5-10 hyphae/field were observed. The membranes carrying the hyphae were transferred to plates of natural soil for incubation periods of 0.5-8 days, then transferred to acidified PDA for 12-24 hr. The fungi on the membranes were then stained. Viability was determined on the basis of renewed growth from the lysed fragments of hyphae,



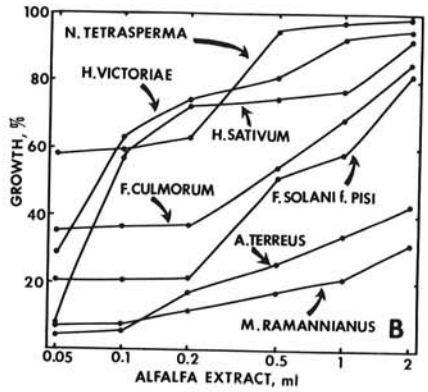


Fig. 1.A) Assay procedure for hyphal growth in soil. **B)** Growth of seven representative fungi in soil amended with different concentrations of alfalfa extract, expressed as per cent of linear growth occurring on alfalfa agar.

and was expressed as per cent of the original number of hyphae.

RESULTS.—*Relationship between length of germ tubes and their growth.*—The effect of initial length of germ tubes on subsequent growth was determined. Conidia of *H. victoriae* and *A. ustus* were germinated on cellophane discs on PDA. After measuring germ tube lengths, the fungus on the cellophane was incubated on sterilized soil. At least 70 hyphae of each fungus were observed. The longer the germ tubes initially, the greater was their subsequent growth. Correlation coefficients were 0.96 for *A. ustus* and 0.84 for *H. victoriae*. For assays of hyphal growth on soil, synchronized germ tubes were selected to reduce this source of variability.

Loss of alfalfa extract in soil.—Attempts were made to estimate the amount of nutrients in soil available for growth of test fungi at 0, 2, 4, 10, 24, and 48 hr after amendment. The levels of water-soluble carbohydrates decreased rapidly. For example, soil amended with 2 ml alfalfa extract contained ca. 410 μg carbohydrates/g soil, as determined by a single extract. After 4, 10, and 24 hr, 290, 120, and 29 μg/g were detected, respectively. With amendments of 1 ml or less, none was detected after 24 hr. Glucose, specifically, was also determined in soil amended with alfalfa extract or with a mixture of glucose and peptone. At zero time, the levels of glucose in soil amended with 0.05, 0.1, 0.2, 0.5, 1, and 2 ml alfalfa extract were 2, 9, 14, 32, 59, and 114 μg/g, respectively, whereas at 4 hr, glucose levels had decreased to 0, 0, 0, 15, 28, and 59 μg/g, respectively. In soil amended with glucose and peptone mixtures (2:1, w/w), the amounts of glucose detected at zero time were 165, 330, 650, 1,650, 3,350, and 6,500 μg/g. After 4 hr incubation, glucose had decreased to 85, 168, 301, 625, 1,275, and 2,650 μg/g, respectively.

Thus, losses of glucose during the 4-hr period of the assays of hyphal growth were ca. 50% of the original amount at all levels of amendment. By contrast, the loss of total carbohydrates in 4 hr was less than 50% at the higher amendments. This indicated that some components of the carbohydrate portion of alfalfa extract are utilized at a more rapid rate than is indicated by loss of total carbohydrates.

Growth of fungal hyphae in amended soil.—Young hyphae of 20 fungi were tested for their ability to

TABLE 1. Mean net growth, relative growth indices, diameter, and growth rates of hyphae; and volumes, germination times, and soil fungistasis sensitivities for spores of various fungi

Fungus	Mean net growth	Relative growth	Hyphal diam	Growth rate	Spore volume	Spore germination time	Spore sensitivity
	μ[a]	*index*[b]	μ[c]	%[d]	μ[e]	*hr*[g]	*index*[h]
Alternaria tenuis	89.0	54.0	2.3	45	1,772	4.0	3.8
Aspergillus fumigatus	84.1	41.9	1.8	41	13	13.0	15.5
Aspergillus terreus	29.3	24.3	1.0	32	8	15.0	23.0
Aspergillus ustus	56.0	63.4	1.8	45	20	8.0	5.8
Botrytis cinerea	28.3	39.1	2.2	37	182	4.0	11.5
Curvularia lunata	127.4	54.8	2.4	47	1,025	1.3	1.5
Fusarium roseum f. sp. *cerealis* 'Culmorum'	111.1	54.8	2.2	44	223	4.0[f]	
Fusarium solani f. sp. *pisi*	75.7	42.3	2.2	45	427	4.0	0.1
Glomerella cingulata	46.1	70.8	2.2	49	212	6.0	3.4
Helminthosporium sativum	170.5	68.1	2.9	53	10,537	2.5	2.5
Helminthosporium victoriae	286.4	77.5	3.5	66	6,333	1.5	0.5
Mucor ramannianus	8.8	20.3	1.6	34	128	10.0[f]	
Myrothecium verrucaria	36.3	39.1	1.8	33	36	6.5	3.8
Neurospora tetrasperma	265.5	82.2	3.8	68	3,839	2.0	0
Pencillium frequentans	9.5	36.8	1.4	31	19	7.0	20.0
Pencillium variabile	9.0	27.0	1.4	33	14	14.0	20.0
Stemphylium sarcinaeforme	110.5	72.4	3.2	52	6,583	3.5	1.3
Verticillium albo-atrum	14.9	34.0	2.1	35	22	12.0	7.5
Thielaviopsis basicola	71.3	71.2	2.1	55	210	5.0	6.2
Trichoderma viride	28.1	38.3	1.7	43	45	17.1	9.1

[a]Mean net growth over the alfalfa extract concentration series. Net growth was the difference between initial hyphal length plus linear growth on nonamended soil, and final length on alfalfa extract-amended soil.

[b]The area under the curve formed by plotting net growth (as per cent of growth on alfalfa extract agar) against log concentration of alfalfa extract in soil, in proportion to the area of the total rectangle, expressed as per cent.

[c]Mean of the tip and basal portions of hyphae measured microscopically (x 970).

[d]$\frac{\log_e L/1}{t}$, where L = length in 4 hr, 1 = the length in 2 hr, e = the natural logarithm, and t = the time interval between initial and final length (2 hr).

[e]Data of Steiner & Lockwood (8).

[f]Datum from this research.

[g]Time for 50% germination on autoclaved soil (8).

[h]Ratio of sterile:natural soil mixture giving 50% germination (8).

grow on soil amended with different amounts of alfalfa extract. All fungi failed to grow when incubated directly on nonamended soil without cellophane, but slight growth of most occurred during incubation on cellophane on nonamended soil. Net growth of each of the 20 fungi was in proportion to the amount of alfalfa extract supplied, although the response differed widely among the fungi. The mean net growth over the concentration series is given for each fungus in Table 1.

Relative growth indices varied from 20.3 to 82.2 (Table 1). Dosage-response curves of seven representative fungi are shown in Fig. 1-B. For example, *N. tetrasperma, H. victoriae*, and *S. sarcinaeforme* had high relative growth indices of 82.2, 77.5, and 72.4, respectively. *Aspergillus fumigatus, M. verrucaria,* and *T. viride* were intermediate, with relative growth indices of 41.9, 39.1, and 38.3, respectively, whereas *M. ramannianus, A. terreus*, and *P. variabile* had low relative growth indices of 20.3, 24.3, and 27.0, respectively. These values can also be interpreted as indicating their sensitivity to soil fungistasis; a high relative growth index indicates low sensitivity to soil fungistasis.

Relationship of relative growth index to other fungal characters.—In an attempt to determine if factors of fungal morphology or behavior might be associated with ability to grow in soil, hyphal diameter and growth rates were determined for the 20 test fungi (Table 1). Hyphal diameter was directly correlated with relative growth index ($r = 0.82$). Hyphal growth rates as determined from the 2nd to 4th hr on alfalfa extract agar were also directly correlated with relative growth indices ($r = 0.91$). Hyphal diameter and growth rate themselves were also directly correlated ($r = 0.88$); thus, fungi with large hyphae tended to grow faster than those with narrow hyphae.

In view of the report of Steiner & Lockwood (8) that fungistasis sensitivity for spores was related to spore volume and germination time, the relationship of their data to relative growth indices for hyphae was considered. Relative growth index was inversely correlated with fungistasis sensitivity index for spores ($r = 0.74$), and with germination times of spores on sterilized soil ($r = 0.70$), and directly correlated with spore volume ($r = 0.73$). Spore volume and hyphal diameter, both of which were related to relative growth indices, were themselves directly correlated ($r = 0.84$). Individual values for these characters are given in Table 1.

Comparison of relative growth indices determined on soil amended with alfalfa extract and glucose-peptone.—To determine whether alfalfa extract might have had some specific effect on the growth of fungi, relative growth indices of seven fungi were determined on soil amended with different amounts of glucose-peptone. Hyphae were measured, following the procedure used for soil amended with alfalfa extract. There was a highly significant correlation ($r = 0.98, P < .005$) between the relative growth indices as determined using both amendments. It was concluded that the response to alfalfa extract was a property of the fungus rather than of the specific nutrient source.

Lysis of ^{14}C-labeled hyphae on soil.—An attempt was made to determine the relationship between relative growth indices and hyphal susceptibility to lysis. Most previous determinations of fungal lysis in soil have been subjective. To devise an objective method for determining lysis, ^{14}C-labeled hyphae of three different fungi were utilized. Equivalent samples were incubated on soil at 24 C for 1, 2, 4, 6, and 8 days. Radioactivity in all three test fungi decreased with increasing time of incubation on soil (Fig. 2-A). The radioactivity lost corresponded to the extent of lysis as determined by direct observation (Table 2). Hyphae of *F. solani* f. sp. *pisi* lysed more slowly than those of *G. cingulata* and *H. victoriae*

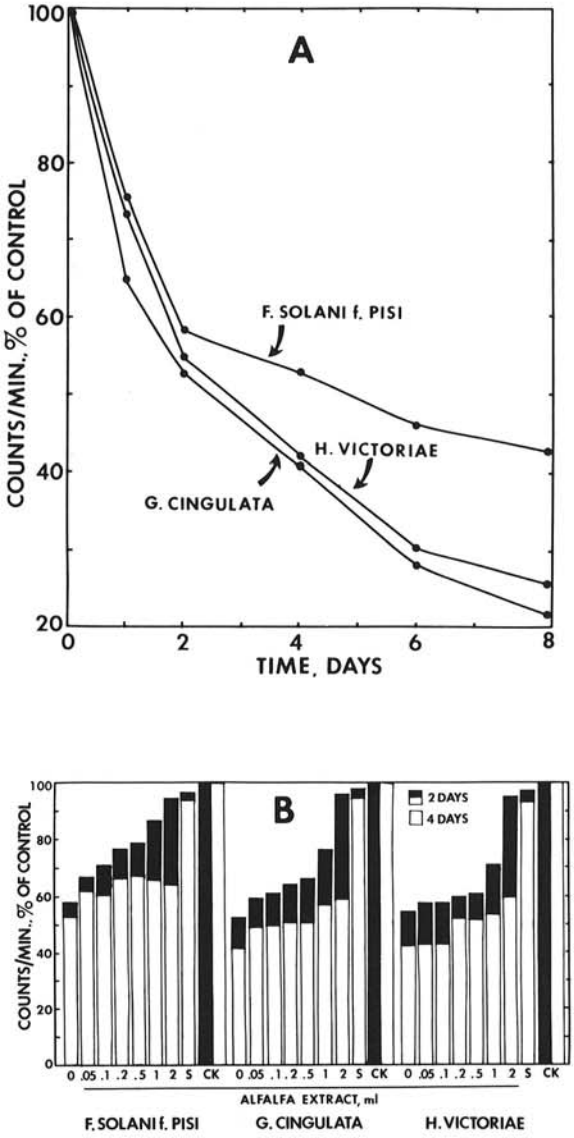


Fig. 2. Lysis of ^{14}C-labeled hyphae on natural soil and soil amended with alfalfa extract. **A)** Loss of radioactivity from ^{14}C-labeled fungal hyphae incubated on soil. **B)** Effect of different amounts of alfalfa extract on lysis of fungal hyphae in soil. S=sterilized soil. CK=control at 0 time.

TABLE 2. Lysis of fungal hyphae incubated on Nuclepore membranes on soil, as determined by loss of radioactive ^{14}C and direct microscopic observation

Day	*Fusarium solani pisi*		*Glomerella cingulata*		*Helminthosporium victoriae*	
	Radioactivity lost, %	Lysis rating[a]	Radioactivity lost, %	Lysis rating[a]	Radioactivity lost, %	Lysis rating[a]
0	0	0	0	0	0	0
1	25	1	35	2	27	2
2	41	2	47	4	45	3
4	47	3	59	4	58	4
6	54	4	72	5	70	5
8	57	4	78	5	74	5

[a]0 = no lysis; 1 = 1 − 10%; 2 = 10 − 30%; 3 = 30 − 70%; 4 = 70 − 90%; 5 = 90 − 99%; and 6 = 100% of hyphae lysed.

(Fig. 2-A), confirming previous results (3, 5). Lysis was delayed when alfalfa extract was added to soil, also confirming previous results (5); the extent of the suppression was greatest with the larger amendments (Fig. 2-B). Differences in amount of lysis occurring over the range of amendment concentrations were greater at 2 days than at 4 days. This could result from the buildup of the microbial population to a higher level with the larger soil amendments, followed by an increased rate of lysis after exhaustion of the amendment. Ko & Lockwood (3) have presented results indicating that lysis of mycelia is due to autolysis induced by microbial deprivation of nutrients in soil. No lysis was observed microscopically on sterilized soil, and the small loss of radioactivity was probably due to respiration.

Although use of ^{14}C-labeled hyphae provided an objective method of measuring hyphal lysis, insufficient data were obtained for comparison with relative growth indices.

Longevity of hyphae and of original germinated spores.—Longevity of hyphae of 10 fungi in soil was determined directly on the basis of renewed growth from lysing hyphal fragments. Hyphae of most fungi survived for at least 24 hr on soil. However, viability decreased with increasing times of incubation after 1 day. There were large differences among the fungi at 2 and 4 days' incubation, but at 8 days fewer than 10% of the hyphae from any test fungi were viable. Partial lysis of hyphae usually resulted in a single viable fragment (Fig. 3-A). However, some hyphae of *H. sativum* formed two-three viable fragments after 2 days' incubation on soil (Fig. 3-B); after 4 days' incubation, these fragments had lysed. Hyphae of most fungi lysed from tip to base, but those of *G. cingulata* lysed from the base toward the tip.

A hyphal survival index was obtained by measuring the area under the curves, relating survival to time and expressing these values as per cent of the total area. Hyphal survival indices were as follows: *F. solani* f. sp. *pisi*, 53; *F. roseum* f. sp. *cerealis* 'Culmorum', 50; *C. lunata*, 47; *H. sativum*, 45; *H. victoriae*, 33; *T. basicola*, 31; *T. viride*, 29; *N. tetrasperma*, 23; *B. cinerea*, 23; and *G. cingulata*, 17. Higher values signify a greater ability of hyphae to survive.

There was no correlation (r = 0.28) between relative growth indices and hyphal survival indices among the 10 fungi tested. Thus, ability to grow in nutrient-amended soil was unrelated to longevity of hyphae in nonamended natural soil. Occasionally, new conidia formed at the tips of lysing hyphae of *H. sativum* (Fig. 3-E) and *H. victoriae*. The new conidia were able to germinate after incubation on acidified PDA (Fig. 3-F). Chlamydospores also developed from lysing hyphae of *F. solani* f. sp. *pisi* and *F. roseum* f. sp. *cerealis* 'Culmorum', and chlamydosporelike structures developed in lysing hyphae of *G. cingulata* after 4 days' incubation (Fig. 4-A, B). These chlamydosporelike structures also germinated after incubation on acidified PDA (Fig. 4-C).

Longevity of the original germinated spore itself in soil was also determined. Spores of some fungi survived longer than their hyphae. After 8 days, 83% of the original conidia of *H. sativum*, 56% of *H. victoriae*, 43% of *C. lunata*, 39% of *F. solani* f. sp. *pisi*, and 35% of *F. roseum* f. sp. *cerealis* 'Culmorum' were still viable, whereas fewer than 10% of their hyphae remained viable. These spores were all multicellular. The single-celled conidia of *T. basicola, N. tetrasperma, T. viride*, and *B. cinerea* lost viability at a rate very similar to that for hyphae. The original single-celled conidia of *G. cingulata* survived for an even shorter period than did their hyphae; lysis always began at the basal portion of the germ tubes, and included the conidia, with the tip of the hyphal fragment persisting longest. Chlamydosporelike structures which formed at the hyphal tip further enhanced the survival ability of the fragments.

The conidia of *H. sativum* and *H. victoriae* were found to remain viable after several germinations. Some of the original germinated conidia were able to regerminate at least 5 times on acidified PDA following complete lysis of hyphae on soil (Fig. 3-C, D; Fig. 5). All germinated conidia of *H. sativum* regerminated at least twice, and 56% regerminated 5 times. Seventy-five per cent of the conidia of *H. victoriae* regerminated twice, and 18% regerminated 5 times. A general relationship between number of cells in a conidium and the number of germinations was observed, but we were unable to determine if each new germination originated from a different cell.

DISCUSSION.—A method was developed to measure saprophytic growth of fungal hyphae on soil amended with different amounts of alfalfa extract. This technique permitted the experimental isolation

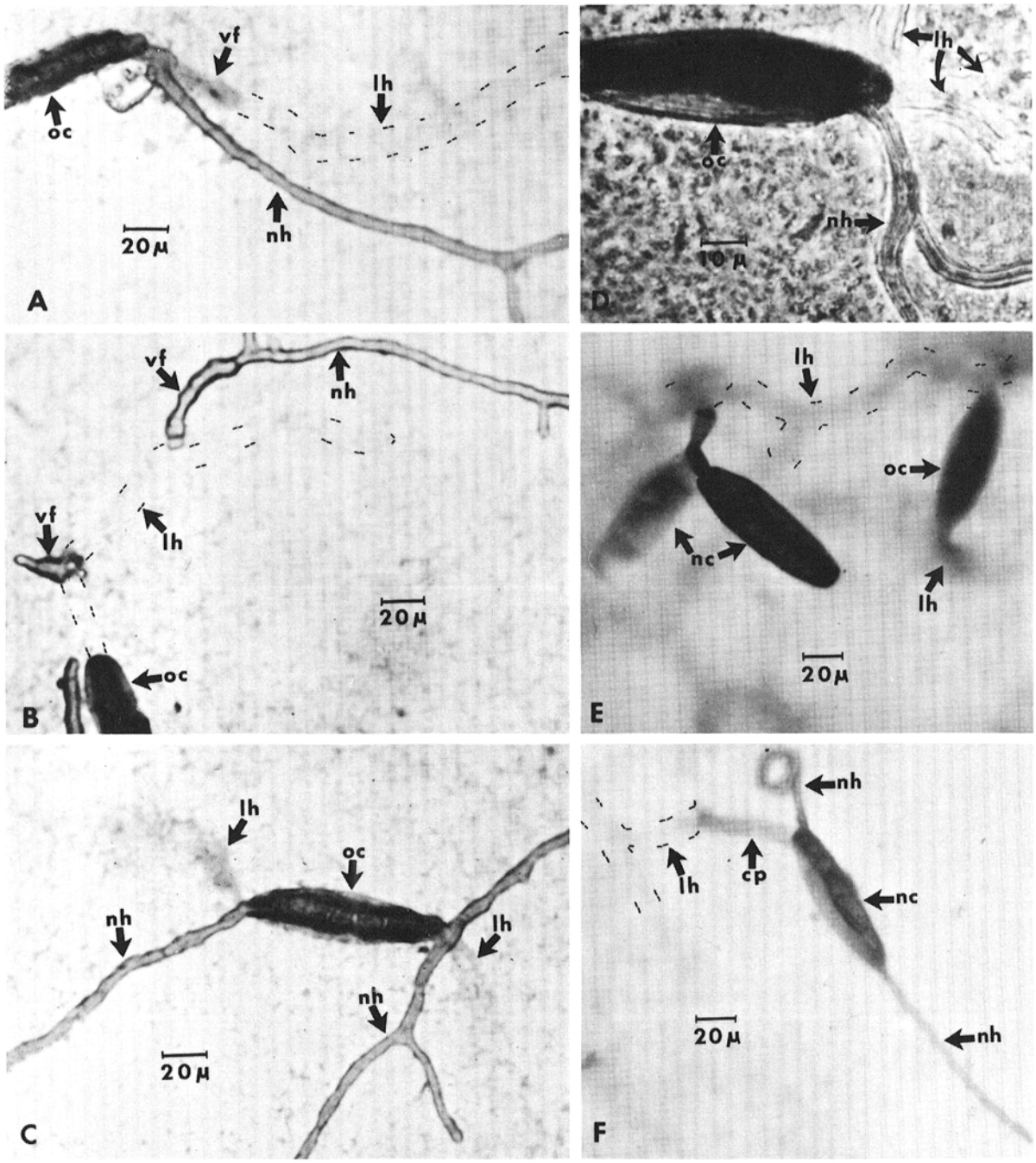


Fig. 3. Lysis and survival of germinated conidia of *Helminthosporium sativum* on Nuclepore membranes on soil. **A)** Single viable fragment growing on acidified potato-dextrose agar (PDA) after partial lysis of the original hypha on soil. **B)** Two viable fragments growing on acidified PDA after lysis of portions of original hypha. **C)** First regermination of the original germinated conidium on acidified PDA after lysis of the two original germ tubes on soil. **D)** Fifth regermination of the original germinated conidium on acidified PDA after lysis of previous germ tubes on soil. **E)** Formation of new conidia from lysed hyphae after incubation on soil for 4 days. **F)** Germination of new conidium from E on acidified PDA. vf = viable fragment; lh = lysed hypha; nh = new hypha; oc = original conidium; nc = new conidium; cp = conidiophore.

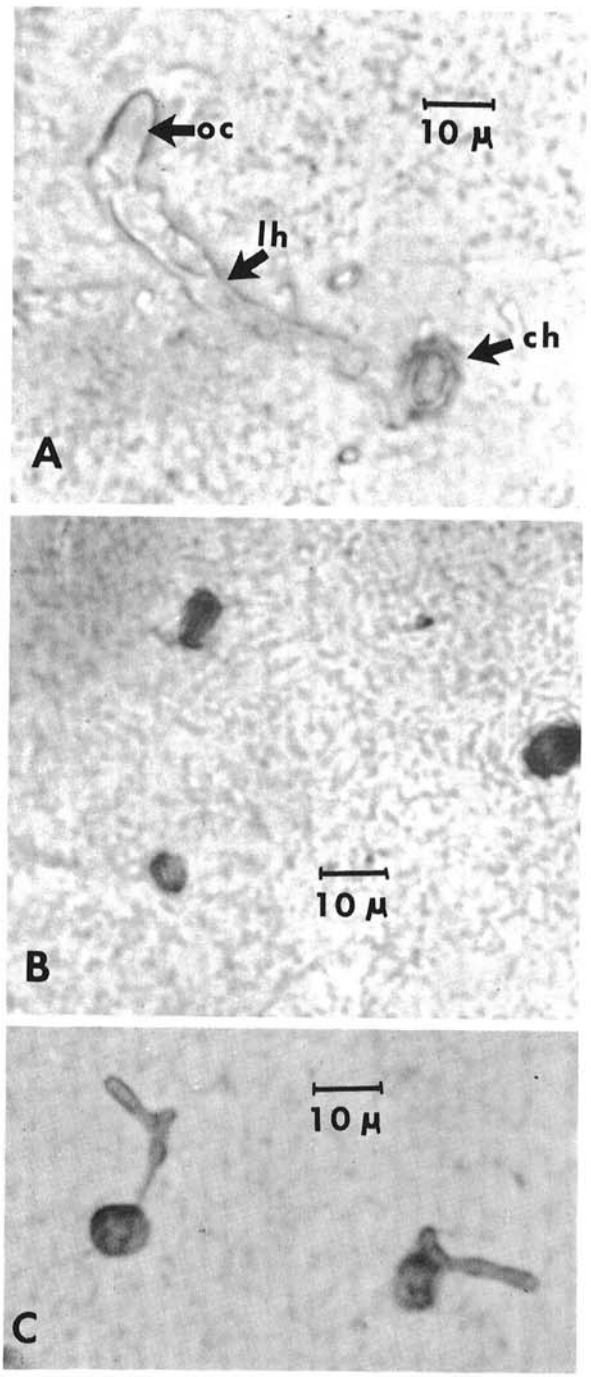


Fig. 4. Chlamydosporelike structures developed in lysing hyphae of *Glomerella cingulata* on Nuclepore membrane on soil. **A)** Development of chlamydosporelike structure from lysed hypha after 4 days' incubation on soil. **B)** Mature chlamydosporelike structures; the original conidia and germ tubes have lysed. **C)** Germination of chlamydosporelike structures on acidified potato-dextrose agar. ch = chlamydosporelike structure; lh = lysed hypha; oc = original conidium.

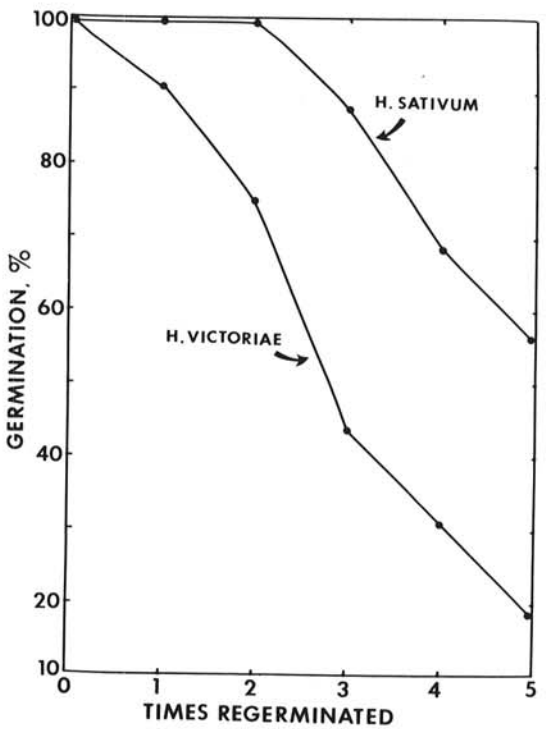


Fig. 5. Regermination ability of original conidia of *Helminthosporium* spp. following lysis of germ tubes, as determined by alternate incubation of germinated conidia on soil and acidified potato-dextrose agar.

of early linear growth in the presence of microbial competition in soil as a character of fungal behavior. In the present work, linear growth was expressed in relation to growth of the same fungus in non-competitive conditions, by use of relative growth indices. This eliminated differences in inherent growth rates of different fungi, and provided a measurement of sensitivity to soil fungistasis.

Our relative growth index is in principle similar to the "reduction factor", a term coined by Garrett (1) to express Wastie's (11) ratio "growth rate under competitive conditions/growth rate under non-competitive conditions" on agar media. In Wastie's work, competitive colonization of agar by 14 root-infecting fungi was directly correlated with growth rate as determined under noncompetitive conditions, but was not correlated with the reduction factor (1, 11). Inspection of Wastie's data also indicates that growth rate and the reduction factor were not correlated. This contrasts with our results, showing a close correlation of growth rate with relative growth index. The discrepancy may be explained by the fact that our measurements were made during the first 4 hr of growth, whereas Wastie's were made much later (11). Garrett (1) has emphasized that the outcome of the struggle for competitive success may be decided early.

The interrelationships among the fungal characters revealed herein and previously (8) (Table 2) suggest that if one aspect of the behavior or morphology of a fungus is known, others might be predicted, within

limits. For example, species with large spores tend to germinate rapidly and exhibit low sensitivity to soil fungistasis (8); they give rise to large hyphae which grow rapidly and which have high relative growth indices. The opposite is true of species with small spores. Thus, fungi may have evolved at least some of their phenotypic characters in an integrated pattern rather than independently.

The reason for the association of low fungistasis sensitivity with large, rapidly germinating spores seems clear, since rapid germination would tend to allow completion of the germination process before depletion of substrate by microbial competition (8). The association of rapid growth rate with large hyphae may also be due to spore size. In the early stages of growth considered, large hyphae may draw a significant portion of their nutriment from the reserves of the spore. The larger relative growth indices possessed by fungi with large, fast-growing hyphae may be due to their being able to exploit a volume of soil more rapidly than the smaller, slower-growing hyphae, and hence gain access to a greater quantity of nutrients before exhaustion.

If the above considerations are valid, the growth responses of the fungi would appear to be explicable in terms of nutrient relations, and do not require an assumption that fungistatic substances are involved. Nonamended soil contained only small amounts of detectable carbohydrates or none, and fungi incubated directly on such soil failed to grow. Growth of all fungi in amended soils was in proportion to the amount of alfalfa extract added. Moreover, the fact that the growth responses were measured very early (0-4 hr) would tend to reduce the chances of antibiotic factors coming into play, as suggested by Wastie (11) and discussed by Garrett (1).

The production of two or more viable fragments from a single hypha during lysis, as occurred with *H. sativum*, might afford some short-term advantage by increasing the inoculum density of soil-borne pathogens which can quickly colonize the rhizosphere. Fungal hyphae are less sensitive to soil fungistasis than are spores from the same species, because hyphae do not require a germination time as do spores (8). Thus, when nutrients are available for fungal growth, as in the rhizosphere, surviving fragments of lysed hyphae may commence growth immediately.

The ability of conidia of *H. sativum* and *H. victoriae* to regerminate when exposed to nutrients after lysis of germ tubes in soil might have long-term survival value. During periods of nutrient deprivation in soil, these fungi appear to be able to conserve energy reserves in their spores until exogenous nutrients become available, when they germinate again. This apparently is the first report of the ability of conidia of *Helminthosporium* to regerminate after lysis of germ tubes.

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